REMARKS

Reconsideration of the subject application is respectfully requested.

Claims 1-7, 9-12 and 17-19 were pending as of the Office Action mailing date of March 28, 2011. This Reply is timely filed within the three (3) month time period for reply set forth in the Action.

Claim 1 has been amended to incorporate limitations, inter alia, of claims 4 and 5. Claims 4 and 5 have been cancelled. Applicant represents and asserts that no new matter is added by any amendments herein.

Applicant notes that rejections over prior art were made in the current Office Action. As such, in view of MPEP 707.07 that requires the action to be complete as to all matters, Applicant proceeds with the understanding that the subject matter has been searched and that the present claims are patentable once distinction is established over the cited prior art.

I. REJECTION UNDER 35 USC 103(a)

Claims 1-7, 9-12, and 17-19 have been rejected under 35 USC 103(a) as being obvious over the cited Su and Faris references of record.

Applicant respectfully traverses this rejection.

The current invention, as now claimed, requires, inter alia:

> a nanopore substrate having a plurality of nanopores and alignment marks, said nanopore substrate having a thickness and said nonapores having a diameter in a range of 50 to 2000 nm resulting in an aspect ratio in a range of 0.5 to 2;
>
> a substantially planar support layer deposited on said nanopore substrate and having a plurality of nanopores corresponding to and aligned with said nanopores of said nanopore substrate;
>
> a biologically effective layer configured to host at least one of a non-lipid molecule and functional molecule, deposited on said support layer and covering the plurality of nanopores, resulting in accessible nanopores from both sides of the biologically effective layer for measurements, wherein the biologically effective layer is a biomembrane isolated from one of prokaryotic and eukaryotic cells, and wherein the biologically effective layer is a lipid bilayer formed by preparation and later fusion of lipid vesicles or is a functional layer of supramolecular assembly, and said biologically effective layer retaining biological functionality. (amended claim 1, emphasis added)

Support for this amendment is found in the specification as filed, page 9, line 4-24 and page 6, lines 19-23.

The claim, as now presented requires thickness of the substrate and diameter of the nanopores arranged to result in an aspect ratio in the range of 0.5 to 2 wherein the nanopores having a diameter in the range of 50 to 2000 nm and biologically effective layer retains biological functionality after fusion of lipid vesicles or is a functional layer of supramolecular assembly.

Applicant acknowledges the current rejection is based on the combined teaching of the two (2) cited references. Applicant will discuss each reference individually and conclude with a discussion of the combined teachings of the cited references.

The cited section of Su in the current office action, paragraph [0072] characterizing Su teaching that "molecules can move through ion channels" is not completely accurate. The relevant ion channels don't let pass **molecules**, but single atomic **ions** such as Na+, K+, Ca++ and so on. In contrast to Su, the present invention provides that not the ion channels itself pass through the nanopore, but single atomic ions in the dependency of the right stimulation of the membrane protein immobilized in the lipid bilayer. In the present invention, it is also possible, for example in case of transporters, that specific molecules can pass through the bilayer proteins that are integrated in the immobilized lipid membranes. Analysis is for the specificity and transport rate of the membrane protein integrated and immobilized the lipid bilayer. This test on specificity and transport rate cannot be achieved by the system of Su.

In the present invention, Ion channels may be incorporated into the assay chip and operably coupled to detectors, which exactly means in the context of the present invention that "Incorporation into chips" is determined as spanning a biological effective layer on nanopores and integrate membrane proteins into the fluid bilayer. The nano-dimensions of the pores as now also incorporated into claim 1 makes this immobilized layer stable enough to host the functional molecule. In case of Su, the labeled molecules **pass through the pores**, as all drawings show,

instead of **immobilizing** them in the lipid bilayer and then check of the response to certain external stimulation by natural ligands and other artificial effector molecules as in the current invention.

A prerequisite of the biological functionality of the integrated and immobilized membrane proteins is a lipid membrane. Measurements also require the immobilization of such lipid bilayers on a surface, whereas the fully integrated (not attached, fixed to the bilayer, see specification as filed p.13, lines 10-36) proteins are in the pore area in the subject invention. Su has no disclosure as to the activity (function) of membrane proteins is measured. Current commercially available patch-clamp instruments provide for a whole cell which is fixed on the surface measure ion translocation as a ion current. In case of the present invention a patch is fixed on a surface allowing measurement in two compartments of unlimited volumes. The device of Su cannot measure the translocation of ions and molecules across lipid bilayers, because the bilayer of Su **is not fixed** and therefore the layer of Su **is not able** to host a non-lipid molecule or functional molecule.

This is in contrast to the present invention in which is described in the specification as filed on page 7, line 3, that states: "This assay chip offers an array of nanopores of macroscopic lateral dimension therefore providing both, supporting area to stabilize the biological effective layers (defined as a layer that preserves the full functionality of the non-lipid molecule hosted therein), such as a lipid bilayer membrane, and pores in a high density in which the biological effective layer remains fully fluid. This assay chip therefore offers a versatile system for various

applications, like drug screening, functional protein analysis, toxicity analysis and the like. Due to the tiny thickness of the Si3N4-layer the respective Si3N4-membrane with the nanopores is also extremely thin and due to the applied fabrication technology these Si3N4-membranes are mechanically stable. The given aspect ratio of the pore diameter to thickness of the nanopore array (now added as limiting feature to claim 1) **allows an un-impeded diffusion of macromolecules to both, the lipid layer membrane and to the non-lipid molecule, such as membrane proteins, integrated therein**. Further, the mechanically stabilized biological effective layer (that means the solid support layer being the Si3N4-membrane with the nanopores and the biological effective layer immobilized thereupon) offers free access from both sides of the biological effective layer what allows the investigation of complex interactions of molecules, such as natural ligands or the interaction with artificial effector molecules (such as drugs) with the functional integrated membrane proteins and to elucidate the mechanism of signal transduction. Due to the accessibility from both sides, the transport of ions, molecules and particles through the biological effective layer by transporter proteins can be investigated in a micro-chamber system, i.e. in a two-compartment system. Furthermore, the membrane proteins are sterically not impeded due to the preservation of their mobility and therefore can directly be investigated on their response to allosteric effects what is crucial for the development of new drugs with GCPRs as the target.

This passage specifies the subject of the present invention. The lipid bilayer of the present invention hosts mandatorily a functional molecule, such as a G-protein

coupled receptor (GDCR), which is then tested against the divers complex interaction of molecules, such as natural ligands or against artificial effector molecules such as drugs. This test can yield for example to the appearance of the conductivity for specific ion which could not pass through the lipid bilayer when the integrated functional molecule is not stimulated properly.

With respect to the additional limitations incorporated now into the independent claim 1, typical diameters of pores of Su are in the range from 1 to 10 nm. These small dimensions are required to detect single ions passing through a channel. Typical diameters according to the present invention range from 50 to 2000 nm. This significant larger dimension is required to immobilize lipid bilayers with integrated proteins in the pore which results then in a complex self-assembled biomolecular system. The patent of Su does not allow to build up such complexes since it has to be kept in mind that the bilayer itself has a thickness of about 5 nm. The high aspect ratio of channels in chips of Su requires an applied voltage for acceleration of the ion transfer as displayed in Fig. 2 and 3 of the Su patent. Free diffusion without voltage application across such channels will be very slow and therefore translocation of ions and molecules across bilayers immobilized on such chips can not be measured. Consequently, Su did not address nor rendered obvious this latter option.

Summarizing the differences between the present invention and Su, biological effective layers according to the invention are biological membranes or lipid bilayers. Immobilization of such layer is a prerequisite for the functional assay. The

patent of Su describes detection of biomolecules passing through a pore which is a completely different principle of detection using a completely different assay chip.

Faris is cited in the current Office Action to teach alignment markings. However, even if, *arguendo*, the alignment teaching of Faris were instructive, the combination of the references into a single instructive disclosure remains deficient because combination would provide a substrate in which **ions** pass, wherein the substrate has alignment markings. This is not the present invention which requires the given aspect ratio of the pore diameter to thickness of the nanopore array and allows an un-impeded diffusion of **macromolecules** to both, the lipid layer membrane and to the non-lipid molecule. Thus, even in combination, the cited references fail to teach or suggest the claimed invention.

Because of the failure of the combined cited references to teach or sugges the current invention, Applicant asserts a rejection under 35 USC 103(a) cannot be properly maintained. Applicant respectfully requests reconsideration and withdrawal of this rejection.

In view of the foregoing, allowance of pending claims 1-3, 6-7, 9-12, and 17-19 is solicited.

Please charge any fees that might be due with respect to Sections 1.16 and 1.17 to

Deposit Account Number 12-1099 of Lerner Greenberg Stemer LLP.

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